Human Oligodendrocyte Characterization Kit

Catalog No. SCR601

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures.

USA & Canada Phone: +1(800) 437-7500 Fax: +1 (951) 676-9209
Australia +61 3 9839 2000

Introduction

Oligodendrocytes are the neuroglial cells of the central nervous system. Their primary function is to produce the myelin sheath which wraps around the axons of neurons and serves as an electrical insulator, allowing for efficient propagation of action potentials[1]. *In vivo*, oligodendrocyte progenitor cells (OPC) arise from the subventricular zones (SVZ) of the lateral ventricle and migrate to the cortex, where they differentiate into mature myelin-forming oligodendrocytes[2]. During development, surface glycoproteins are expressed at distinct time points and often play dual roles in regulating differentiation and myelin biogenesis[3].

EMD Millipore's Human Oligodendrocyte Characterization Kit (SCR601) contains validated antibodies that characterize different developmental stages of oligodendrocytes along with two antibodies for the detection of neurons and astrocytes, respectively. The kit includes:

- Rabbit anti-NG2 Chondroitin Sulfate Proteoglycan: Early oligodendrocyte progenitor marker[4].
- Mouse anti-Myelin Proteolipid Protein and DM20 (PLP/DM20): Intermediate to late oligodendroctye marker.
- Mouse anti-Galactocerebroside C (GalC): Intermediate to late oligodendrocyte marker. Major component of the myelin sheet.
- Mouse anti-Myelin Oligdodendrocyte Glycoprotein (MOG): Late oligodendrocyte marker. Can be used to identify functional oligodendrocytes.
- Mouse anti-MAP2, clone AP20: stains neurons.
- Mouse anti-Glial Fibrillary Acidic Protein, clone GA5 (GFAP): stains astrocytes.

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Kit Components

1. Rabbit anti-NG2 Chondroitin Sulfate Proteoglycan: (Cat. No. CS204510): One vial containing 10 μg polyclonal antibody. Store at -20°C

2. Mouse anti-Myelin Proteolipid Protein, C-terminus, clone PLPC1: (Cat. No. CS204475) One vial containing 20 μg monoclonal antibody. Store at -20°C.

3. Mouse anti-Galactocerebroside, clone mGalC: (Cat. No. CS204476) One vial containing 10 μg monoclonal antibody. Store at -20°C.

4. Mouse anti-Myelin Oligodendrocyte Glycoprotein (MOG): (Cat. No. CS204477) One vial containing 10 μg monoclonal antibody. Store at 2-8°C.

5. Mouse anti-Glial Fibrillary Acidic Protein, clone GA5: (Cat. No. CS204478) One vial containing 10 μg monoclonal antibody. Store at 2-8°C.

6. Mouse anti-MAP2, clone AP20: (Cat. No. CS204479) One vial containing 10 μg monoclonal antibody. Store at 2-8°C.

Materials Required but Not Supplied

1. Tissue culture-wares and supplies
2. Laminin (Catalog No. CC095)
3. Poly-L-ornithine (Sigma Catalog No. P4957)
4. Millicell EZ SLIDE 8-well glass, sterile (Cat. No. PEZGS0896)
5. Human Oligodendrocyte Progenitor Cells (Cat. No. SCR600)
6. Human OPC Expansion Media Kit (Cat. No. SCM107)
7. Human OPC Differentiation Media Kit (Cat. No. SCM106)
8. Accutase™ Cell Dissociation Solution (Cat. No. SCR005)
9. Phosphate-Buffered Saline (1X PBS) (Cat. No. BSS-1005-B)
10. EmbryoMax ES Cell Qualified Ultra Pure Water, sterile H20, 500 mL (Cat. No. TMS-006-B)
11. Fixative (e.g. 4% Paraformaldehyde in 1X PBS)
12. Blocking Solution (5% normal donkey serum, 5% BSA ±0.1% Triton X-100 in 1X PBS)
13. Non-Permeable Blocking Solution (5% normal donkey serum in 1X PBS)
14. Mouse IgG, purified 10 mg (Catalog No. PP54)
15. Rabbit IgG, purified 25 mg (Catalog No. PP64)
16. Fluorescent-labeled secondary antibodies. Donkey anti-mouse IgG, Cy3 conjugated (Cat. No. AP192C), donkey anti-mouse IgG, FITC conjugated (Cat. No. AP192F), and donkey anti-rabbit IgG, Cy3 conjugated (Cat. No. AP182C) are recommended.
17. Trypan Blue
18. Anti-fading mounting solution (DABCO/PVA)
19. 4'-6-Diamidino-2-phenylindole (DAPI) / PBS solution.
20. Scepter™ Handheld Automated Cell Counter (Catalog No. PHCC00000) or Hemacytometer
21. Microscope with appropriate fluorescent filters

Storage

When stored at the recommended storage conditions (refer to Kit Components), components are stable up to the expiration date. Do not expose to elevated temperatures and avoid repeated freeze/thaw cycles. Discard any remaining reagents after the expiration date.

Immunostaining Protocol (for 8-well chamber slides)

1. Fix the cells by incubation in 2% paraformaldehyde in 1X PBS for 10 minutes at room temperature.

2. Carefully aspirate the fixative and rinse four times (5-10 minutes each) with 1X PBS.

3. Prepare the blocking solution (e.g. 5% BSA, 5% normal donkey serum In 1X PBS). For intracellular staining, add 0.1% TX-100 to the blocking solution to permeate the cells.

4. Carefully aspirate the 1X PBS wash and add the blocking solution. Incubate at room temperature for 2 hours or overnight at 4°C. **IMPORTANT: Do not shake the cells**.

5. Dilute the primary antibodies to working concentrations in the appropriate blocking solutions. For optimal results, the following antibody dilutions are recommended for immunocytochemistry.

Table 1. The recommended antibody list for the characterization of Human OPCs and their differentiated progenies is presented below.

Marker	**NG2**	**PLP/DM20**	**GalC**	**MOG**	**MAP2**	**GFAP**
Catalog No.	CS204510	CS204475	CS204476	CS204477	CS204479	CS204478
Recommended Dilution	2.5 to 5 (μg/mL)	10 (μg/mL)	2.5 to 5 (μg/mL)	10 (μg/mL)	2.5 to 5 (μg/mL)	2.5 to 5 (μg/mL)
Subcellular localization	Surface	Surface	Surface	Surface	Intracellular cytoskeleton	Intracellular cytoskeleton

6. In a separate control well, depending upon the specific antibody used, add equivalent concentrations of mouse IgG (1 mg/mL) or rabbit IgG (1 mg/mL) to 0.5 mL of the appropriate blocking solution. For example, to obtain a 1/200 dilution of mouse anti-GalC (1 mg/mL), 2.5 μL of the antibody is added to 0.5 mL volume of the blocking solution. In an adjacent control well, add 2.5 μL mouse IgG (1 mg/mL) control antibody to 0.5 mL of the blocking solution.

7. Carefully remove the blocking solution from each well and add the appropriate diluted primary antibodies to each well. Incubate at room temperature for 4 hours, or 2 to 8°C overnight. **IMPORTANT: Do not shake**.

8. Remove the primary antibody solution. Wash the cells four times with blocking solution (5 minutes each wash).

9. Dilute secondary antibodies to 1 to 5 μg/mL concentration (1:200 to 1:1000 dilution) with the blocking solution.

10. Remove the last wash and add the appropriate diluted secondary antibody to each well. Incubate at room temperature for 1 hour. Cover the plate with tinfoil to protect from the light.

11. Remove the secondary antibody solution. Wash 4 times (5 minutes each) with 1X PBS.

12. Prepare DAPI dye: dilute DAPI with 1X PBS to 1μg/mL (1:10,000 dilution).

13. Remove the last wash; add DAPI staining solution and incubate at room temperature for 15 minutes.

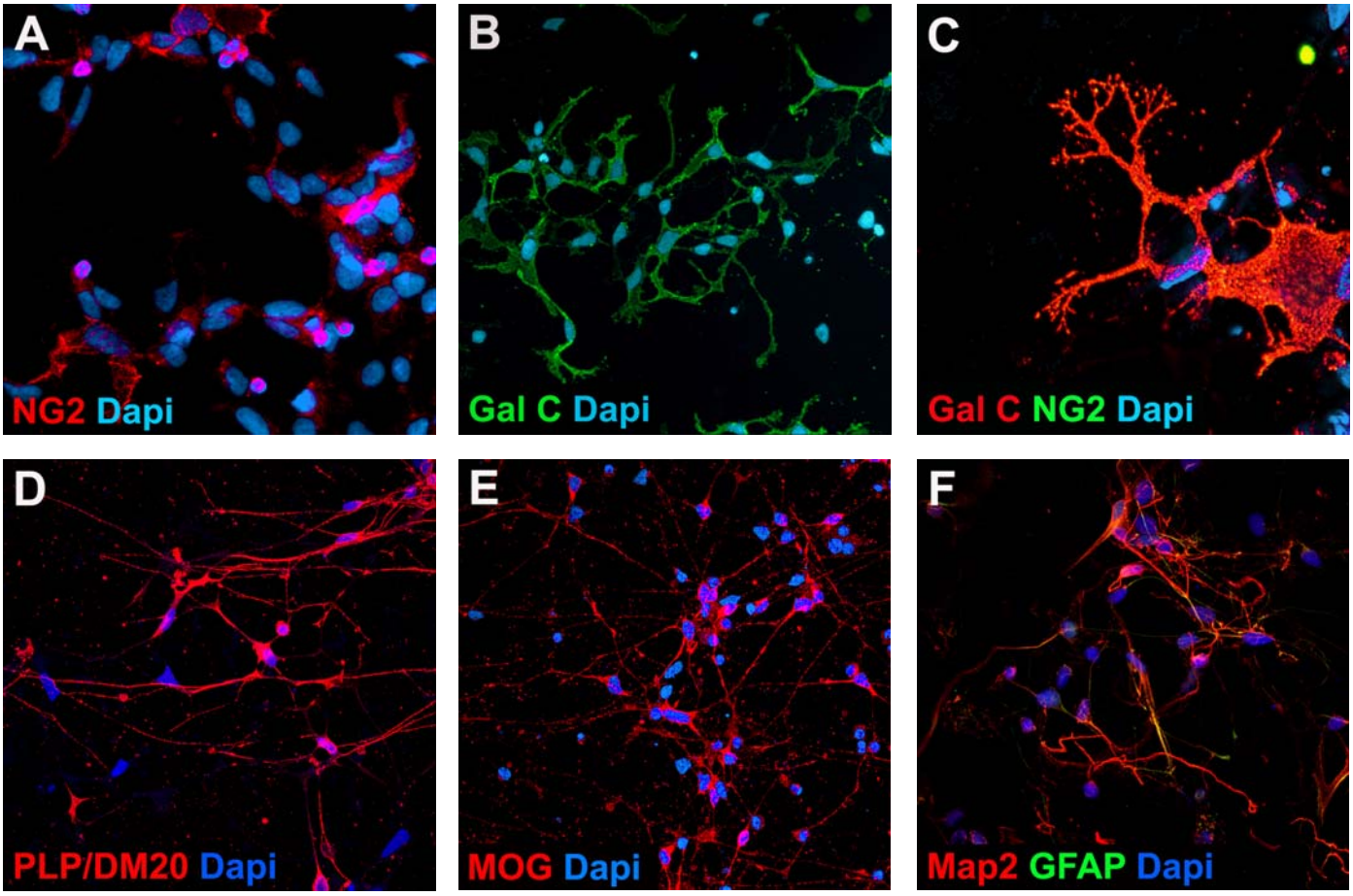
14. Remove the DAPI solution; wash twice with 1X PBS.

15. Mount a glass coverslip over the chamber slides using anti-fading mounting solution (e.g. DABCO/PVA).

16. Visualize the cell staining with a fluorescent microscope. ***Note:*** *Be sure to use the correct filter to visualize fluorescent-labeled cells*.

Result

The antibodies included in the Human Oligodendrocyte Characterization kit (SCR601) have been validated on human oligodendrocytes cultured in *in vitro* culture. Similar analyses in other species have not been tested.



Characterization of EMD Millipore's Human Oligodendrocyte Progenitor Cells (OPCs) (Catalog No. SCR600) and their differentiated progenies. Proliferating Human OPCs express NG2 (**A**) and GalC (**B**). After two weeks of spontaneous differentiation, approximately 30% cells become mature oligodendrocytes that express NG2, Gal C (**C**), PLP/DM20 (**D**), and MOG (**E**) while ~50% cells differentiate into MAP2 expressing neurons (**F**) with very little astrocytes (GFAP, **F**) detected. Human ES cell derived oligodendrocyte progenitor cells (Cat. No. SCR600) were plated at $10^4/cm^2$ onto poly-L-ornithine (10 μg/mL) and laminin (10 μg/mL) coated 8 well chamber slides in Human OPC Expansion Complete Media (Cat. No. SCM107). Twenty-four hours post-seeding, spontaneous differentiation was initiated by media exchange with Human OPC Spontaneous Differentiation Complete Media (Cat. No. SCM106).

Related Products

The following products are available from Millipore as separate items:

1. Human Oligodendrocyte Differentiation Kit (Cat. No. SCR600)
2. Human OPC Expansion Media Kit (Cat. No. SCM107)
3. Human OPC Spontaneous Differentiation Media Kit (Cat. No. SCM106)
4. Mouse anti-GalC antibody, 50 μg (Cat. No. MAB342)
5. Mouse anti-PLP/DM20 antibody, 100 μg (Cat. No. MAB388-100UG)
6. Mouse anti-MOG antibody, 100 μg (Cat. No. MAB5680)
7. Mouse anti-MAP2 antibody, 200 μg (Cat. No. MAB3418)
8. Mouse anti-GFAP antibody, 40 μg (Cat. No. MAB3402)
9. Mouse anti-GFAP antibody, Alexa488® conjugated, 100 μg (Cat. No. MAB3402X)
10. Rabbit anti-NG2 antibody, 100 μg (Cat. No. AB5320)
11. Mouse anti-CNPase antibody, 100 μg (Cat. No. MAB326)
12. Mouse anti-MBP antibody, 50 μg (Cat. No. 05-675)
13. Rabbit anti-Sox10 antibody, 100 μg (Cat. No. AB5727)
14. Mouse anti-O4 antibody, 50 μg (Cat. No. MAB345)

Reference

1. Baumann, N. and Pham-Dinh, D. (2001) Biology of oligodendrocyte and myelin in the mammalian central nervous system. *Physiol Rev.* **81(2)**: 871-927.

2. Mallon, B. S, Shick, H. E., Kidd, G. J., and Macklin, W. B. (2002) Proteolipid promoter activity distinguishes two populations of NG2-positive cells throughout neonatal cortical development. *J. Neurosci.* **22(3)**: 876-85.

3. Jackman, N., Ishii, A., and Bansal, R. (2009) Oligodendrdocyte development and myelin biogenesis: parsing out the roles of glycosphingolipids. *Physiology (Bethesda)* **24**: 290-7.

4. Trotter, J., Karram, K., and Nishiyama, A. (2010) NG2 cells: properties, progeny and origin. *Brain Res Rev.* **63**: 72-82.

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